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STUDIES ON THE TUBE-BUILDING AMPHIPOD *COROPHIUM TRIAENONYX* STEBBING FROM VISAKHAPATNAM HARBOR: EFFECT OF SALINITY AND TEMPERATURE

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Factors such as salinity and temperature that control the distribution of fouling organisms are of considerable interest. Seasonal variations in salinity are of great importance in the distribution of such organisms because at some places the salinity may be sufficiently high for their survival only during certain months. It would appear that relatively few studies have been made to determine the effect of wide ranges of salinity. Beadle and Cragg (1940) carried out some experiments on the salinity tolerance of *Gammarus* and more extensive work was completed by Kinne (1952, 1953a, 1953b, 1956, 1959 and 1960) on the tolerance of *Gammarus duebeni* and *Gammarus salinus*.

The importance of temperature to stenothermal organisms is well known. Some observations have been made here on the influence of temperature on *Corophium triaenonyx*. Kinne (1952, 1953a, 1953b, 1954, 1956, 1959 and 1970) dealt with the heat tolerance of the amphipod *Gammarus duebeni*. Resistance of the amphipods *Hyalella azteca, Gammarus fasciatus* and *Gammarus pseudolimnaeus* to lethal temperatures was studied by Sprague (1963). Every individual has an optimum temperature or upper biokinetic limit above which activity may suddenly cease in what is known as heat coma. Heat coma and death occur in sequence and may not be simultaneous. Low temperatures can effect animals in a similar way.

A study was undertaken to find out the effects of salinity and temperature on the tube building amphipod *Corophium triaenonyx* at Visakhapatnam. Such fouling organisms are known to withstand wide ranges of salinity and temperature. Investigations were therefore undertaken to determine the maximal and minimal salinity and temperature values at which the adults and juveniles of *C. triaenonyx* survive, to study their reactions to gradual changes in salinity and temperature and to observe the tolerance of animals *in situ*.

During the period of study the overall salinity level of the harbor waters showed wide fluctuations. The highest salinity values recorded were 34.67‰ and 34.62‰ and 34.62‰ in the years 1961, 1962 and 1963, respectively. The lowest salinity values for these years are 14.15‰, 14.66‰ and 8.66‰. The maximum temperatures of the water were 30.80° C, 30.80° C and 30.84° C in the years 1961, 1962 and 1963, respectively.

Materials and Methods

For all experiments on salinity tolerance, normal seawater was diluted with glass distilled water as desired. A series of solutions covering a range 8‰ (lowest recorded in the field) 7‰, 6‰, 5‰, 4‰, 3‰, 2‰, 1‰ down to zero were prepared by dilution. For temperature experiments, finger-bowls containing 200 ml of seawater of salinity 29.86‰ were kept in thermostats at desired temperatures and aerated at intervals. Several experiments were conducted at a time using different thermostats with temperatures ranging from 30° C–40° C.

Twenty animals of both sexes and approximately of the same size groups were used in each experiment and each experiment was repeated at least six times. Animals carefully removed from their tubes were directly transferred from seawater of salinity 29.86‰ and temperature 30.5° C to each of the fingerbowls with experimental media and experimental temperatures. At regular intervals the water was aerated and the animals were observed under a binocular microscope. Dead animals were removed. Emphasis was placed on actual survival rather than on their ability to carry on normal activity. The salinity and temperature at which 50% of the animals failed to recover in a 12-hour period as judged by their inability to respond to needle prickings was considered lethal (Evans 1948). For all experiments controls were maintained. For all salinity experiments a constant temperature of 30.5° C was used and for all temperature experiments a salinity of 29.86‰ was maintained.

Results

Decreased salinity

An initial experiment was designed to find out the maximum salinity at which *C. triaenonyx* survives and also to determine how well they might recover after exposure to abnormally low salinities. In distilled water they die in one hour and forty-five minutes. Animals subjected to salinities between 4‰ and 10‰ died after 6 days and those between 10‰ and 40‰ survived for more than 8 days. The lower lethal salinity lies below 4‰. The survival period in the control bowls was 12–14 days. Another set of experiments was conducted with concentrations from 4‰ down to distilled water. Animals survived for 2 days in 1‰ concentration but they were found to be inactive. So it may be concluded that the minimum salinity for survival activity is 4‰. A series of experiments consisting of concentrations from 1‰ to 0.5‰ was prepared to fix the lower lethal salinity. The results are shown in Figure 1. Death occurred at 0.6‰ concentration. Thus it becomes clear that the lower lethal salinity of *C. triaenonyx* is 0.6‰. At 4‰ and above they remain very active. Of course such low salinities may seldom occur in nature. In the present experiments the fall in salinity is sudden, and more rapid than could possibly occur in the harbor.

Increased salinity

The annual maxima of salinity as observed in the harbor were 34.67‰, 34.62‰ and 34.62‰ for the three years. Animals were placed in solutions of seawater of increased strength from 40‰ to 55‰ adopting the methods described in the foregoing experiments. The different concentrations were prepared by addition of appropriate quantities of sodium chloride. They were able to survive in 55‰ concentration for three days. A second set of solutions was prepared with concentrations ranging from 55‰ to 58‰. In 58‰ they lived for 24 hours. A third

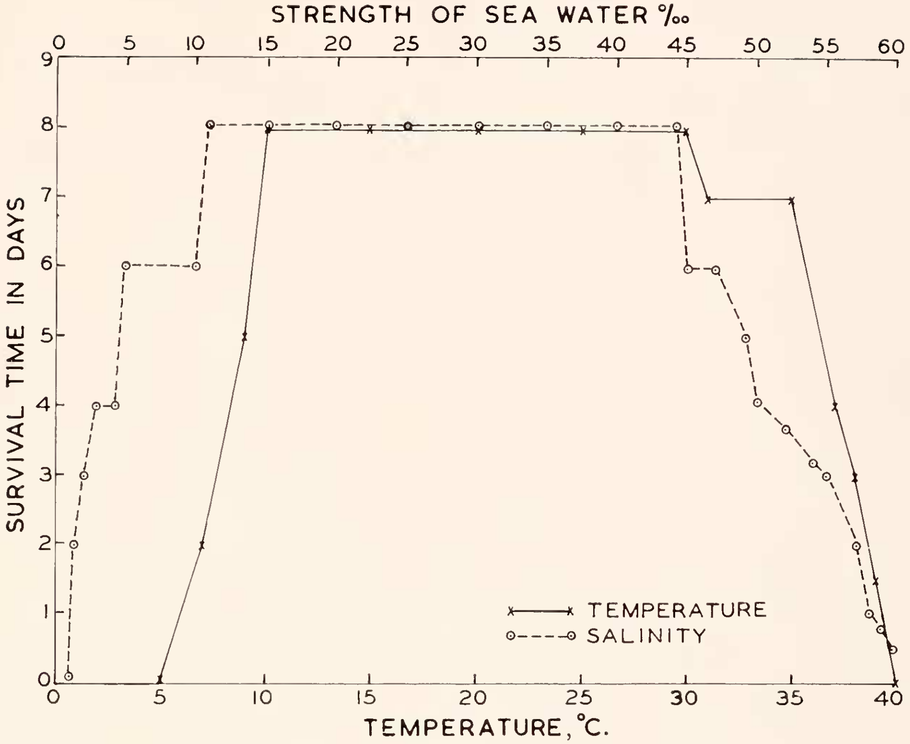

FIGURE 1. Tolerance of *C. triacnonyx* to increased and decreased salinities and temperatures.

set of experiments with solutions consisting of closely graded concentrations ranging from 58‰ to 60‰ at 1‰ intervals. The results are presented graphically in Figure 1. The upper lethal salinity has been found to be 59.8‰, in which concentration immediate death occurred. In media up to 55‰ animals moved about actively and of this concentration and below the animals live quite normally. From all these results it is clear that *C. triacnonyx* is a very hardy form tolerating a wide range of salinity from 0.6‰ to 59.8‰.

Reactions to gradual changes

Some experiments were conducted to assess any differences in tolerance to gradual increase or decrease in salinities. The animals were kept in seawater of salinity 29.86‰ for 24 hours and then they were transferred to the desired decreased or increased concentrations. Procedures described in the prevoius experiments were followed in preparing higher or lower concentrations and the same precautions were taken for aerating the water and removing dead animals as in the previous case. It was found that the survival time is not increased by gradually raising or lowering the salt content of the sea water.

Some experiments were conducted with animals in their tubes. No differences could be found in survival times in the salinity gradients.

Extreme limits of tolerance to temperatures

In all experiments the animals were transferred directly from 30.5° C to the experimental temperatures. At 37° C they lived quite normally for four days and then death ensued. The animals could survive for 10–12 hours at 39° C. At 40° C all the animals succumbed in 2 hours. Thus it could be established that *C. triaenonyx* tolerates wide ranges of temperature up to 40° C but sudden change to unnatural temperature has some effect.

Reactions to gradual changes

In the second set of experiments the conditions and precautions were the same as in the previous experiments. The specimens were placed in a finger bowl consisting of 100 ml of sea water and placed in a bath adjusted to 31° C which is the maximum temperature recorded in the field. Then the temperature was increased by 1° C every day. The temperature of the water in the bowl and the outside air were regularly noted. It was found that with gradual increase in temperature the animals can remain active up to 42.5° C. Beyond this point activity ceased, and at 46° C they were all dead. However with immediate gradual cooling from 42.5° C recovery was possible and the animals once more were observed to be active. Irreversible heat coma apparently sets in from 42.5° C and above (Fig. 1).

A third experiment was conducted on the same lines with a minor change. The temperature was increased by 1° C every hour instead of at 24-hour intervals. In this case the animals become comatose at 42.5° C and at 44° C they appeared dead. It would appear that higher lethal temperature for *C. triaenonyx* lies at 44° C, when the temperature is raised by 1° C every one hour. Some of the animals were removed from the thermostat bath at 42.5° C and brought to room temperature. After 18 hours of cooling some of them recovered. Animals which were held at 44° C or above could not recover.

Some experiments were also conducted with animals in tubes and the following results were obtained. With sudden increase they survived up to 43° C and with gradual increase of 1° C every day up to 48° C and 1° C every hour up to 46° C.

It is clear that the lethal temperatures lie at slightly higher level when the animals are inside the tubes.

Although experiments at lower temperatures were not completed, it is suggested that the lower lethal temperature of *C. triaenonyx* lies at 5° C. Acclimation experiments in this direction could not be conducted.

Effects of salinity and temperature combinations

Kinne (1952, 1953a, 1954 and 1964) made an extensive study of salinity and temperature combinations on *Gammarus duebeni*.

In the present study 6 temperature and 9 salinity combinations were chosen. Twenty specimens were used in each experiment. The salinities and temperatures tested have been 1‰, 5‰, 15‰, 20, 25‰, 30‰, 35‰ and 40‰, and 8° C, 15° C, 22° C, 30° C, 35° C and 39° C respectively. Other conditions were the same as in previous experiments and dead animals were promptly removed in the course of the experiments. The results are given graphically in Figure 2.

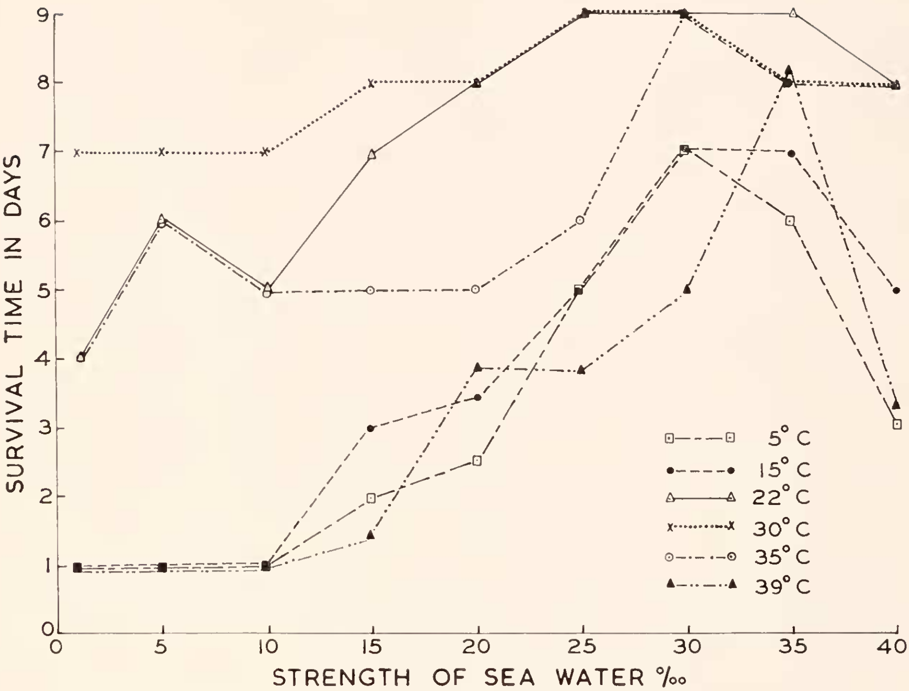

FIGURE 2. Effect of salinity and temperature combinations on *C. triaenonyx*.

It thus becomes (Fig. 2) that *C. triaenonyx* tolerates lower salinities better at higher temperatures, and also the animals are able to tolerate the lower temperatures well when the salinities are nearer normal values.

Effects of size and sex

Size and sex do not seem to have any influence on tolerance. Young ones could be equated to the adults in respect of their tolerance to temperature and salinity. No clearcut correlation between sex and resistance could be made out. However according to Kinne (1953a and 1953b) males are less sensitive to higher temperatures. In the present experiments young females and males appeared to be more resistant than the adults.

DISCUSSION

It is well known that many marine animals cannot tolerate wide ranges of salinities in their environments. However, gradual increase or decrease in salinity may pass unnoticed. *Corophium triaenonyx* is a hardy form and has been found to tolerate wide ranges in salinity. It is able to survive in distilled water for nearly two hours although a flatworm like *Monocelis fusca* dies immediately. (Rees 1941). The present observations demonstrate that this amphipod has a greater resistance to fluctuations. Its lower lethal salinity is 0.6‰ and the upper lethal is 59.8‰.

Under natural conditions, it can survive a rise of 27‰ (from 8‰ to 35‰). The flatworm *Monocelis fusca* can also tolerate wide ranges of salinity, although its behavior in increased salinity is somewhat different from that at lower values (see Rees, 1941). In Kinne's experiments on *Gammarus duebeni* (Kinne, 1959) it was found that all specimens remained motionless when salinity rose to 85‰, but that they regain activity upon transfer to normal conditions. However, they could not remain alive in 85‰ for more than 24 hours.

Gradual acclimation to higher temperature, with a resulting increase in the upper temperature tolerance for the species, has been demonstrated by McLeese (1956) with the lobster *Homarus americanus*. Acclimation to higher temperature also increases the lethal temperature levels for two intertidal crabs, *Hemigrapsus nudus* and *Hemigrapsus oregonensis* (Todd and Dehnel 1960).

Different species have different capacities for temperature acclimation. There is clear proof that when the temperature is increased gradually by 1° C each day, survival at higher temperatures is greatly increased in *C. triaenonyx*. Heat tolerance could be increased in lobster *Homarus americanus* when transferred from 14.5° C to 23° C with about 22 days for total acclimation (McLeese, 1956). Acclimation at high temperatures raised the lethal levels of temperature in *Hemigrapsus nudus* and *Hemigrapsus oregonensis* (Todd and Dehnel, 1960). Acclimation at 20° C increased the lethal level for *Gammarus fasciatus* and *Gammarus pseudolimnaeus* (Sprague, 1963). There was no change in the heat tolerance of *Corophium triaenonyx* during different periods of the year. Edwards and Irving (1943) found a 10° C difference between summer and winter conditions in the death point of the sand crab *Emerita talpoida*.

Temperature stress in conjunction with salinity has been studied less extensively in amphipods. In the present investigation, *Corophium triaenonyx* tolerates lower salinities better at higher temperatures and lower temperatures at more normal salinities. Broekema (1941) investigated temperature and salinity effects on the shrimp *Crangon crangon*. This shrimp was shown to endure lower salinities better at higher temperatures. Its optimal salinity at 4° C is 33‰ and between 20° C and 22° C it is 28–29‰. McLeese (1956) has demonstrated that the lobster *Homarus americanus* has a better survival when both temperature and salinity were high. Temperature can change the salinity range, and salinity can change the temperature range of a species. Todd and Dehnel (1960) have shown that higher temperatures together with higher salinity were most favorable for *Hemigrapsus nudus* and *Hemigrapsus oregonensis*. A temperature-salinity relationship was shown to exist in the crab *Rithropanopeus harrisii* (Kinne and Rotthauwe, 1952).

Size and sex do not seem to have any influence on the tolerance of *Corophium triaenonyx*. The effect of size and sex on tolerance to heat does vary in other animals. Edwards and Irving (1943) found no difference in tolerance in *Emerita talpoida* between males and females but larger animals seemed more resistant than the smaller ones. McLeese (1956) observed that, in the size-groups of lobster studied (21–28 cm), there is identical response to upper lethal temperatures. (Kinne 1959) showed a lower tolerance in *female* amphipods (*Gammarus duebeni*) to higher temperatures, and higher tolerance in smaller individuals of both sexes. Sprague (1963) observed that resistance to higher temperatures decreased with size in *Gammarus fasciatus*, and that female gammarids were more resistant than the males.

It is clear from the experimental results reported here that *Corophium triaenonyx* is hardy both from salinity and temperature points of view. Gradual acclimations over long intervals of time gave a better survival values. For instance when the temperature was increased by 1° C every day they were able to tolerate 46° C. Field data for its natural environment show fluctuations for salinity (8.66‰–34.67‰) and for temperature (26.4° C–30.84° C). The experimental results demonstrate that *C. triaenonyx* in the field lives well within the ranges of tolerance both for salinity and for temperature. However, in an area like Visakhpatnam harbor, factors other than salinity and temperature may also have to be considered. Visakhapatnam harbor receives sewage materials and effluents from industries like the Caltex Oil Refinery and Coramandel Fertilizers, and various other categories of pollution depending upon the nature of such cargo vessels as are berthed there from time to time.

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Summary

1. Investigations were undertaken to determine the maximal and minimal salinity and temperature values at which the adults and juveniles of *Corophium triaenonyx* survive, to study the effect of gradual changes, and also to observe the temperature and salinity ranges of natural populations in the environment.

2. This amphipod tolerates wide ranges of salinity from 0.6‰ to 59.8‰, which are the lower and upper lethal salinities respectively. Between 4‰ and 55‰ they live quite normally. Gradual increase or decrease in the salt content does not seem to have any effect on survival time. Animals *in situ* behaved in a similar way to those out of the tubes.

3. *C. triaenonyx* can tolerate temperature ranges from 5° C to 40° C. Survival times increase when the animals are subjected to move gradual changes. When the temperature is raised by 1° C every 24 hours they could survive up to 46° C. When the temperature is raised by 1° C every one hour they died at 44° C. Animals within their tubes seem to be more tolerant.

4. These amphipods tolerate lower salinities better at higher temperature, and lower temperatures better when the salinities are nearer normal values. Size and sex do not seem to have any influence on the survival time.

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